



Effect of autonomic blockade on ventricular repolarization shortening: Response to behavioral stimulus in paced dogs

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Abstract

Autonomic tone has been suggested to be a significant determinant of ventricular repolarization duration with both rate dependent and independent effects. Using the His bundle-paced dog, a model that eliminates the need for QT correction factors, we explored the rate-independent effects of sympathetic and parasympathetic blockade on ventricular repolarization shortening following an excitatory stimulus. Six male His bundle-paced beagle dogs were paced at 80 bpm and fitted with jackets, surface ECG electrodes, and radiotelemeters. Dogs were given propranolol, atropine methyl nitrate, or the appropriate control in a four-period crossover design. Doses were based on literature reviews and unpublished pharmacokinetic/pharmacodynamic modeling to provide efficacious beta- and parasympathetic blockade throughout the data collection period. Data collection began at 11 am and concluded at 11 am the following day, with event stimuli provided by investigators entering the room at 5 pm and at 7 am the following morning. One minute of ECG data were sampled every 15 min and these means were averaged to generate hourly means for the 24 hour data collection period. Treatment with atropine attenuated RT interval shortening when compared with the vehicle group at both the 5 pm and 7 am stimulus. In contrast, propranolol was not associated with significant effects on RT interval duration at either time point. These results suggest that parasympathetic withdrawal is the primary factor responsible during both awake hours (5 pm) and in the transition from deep sleep to the awake state (7 am) in the facilitation of RT interval shortening following an excitatory stimulus. The attenuation of RT interval shortening following atropine treatment may be a direct effect, or an indirect effect requiring an excited state to become evident. The use of a model that eliminates the need to apply correction factors to repolarization indices helps to clarify the role of the autonomic nervous system on ventricular repolarization.

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1. Introduction

Cardiac repolarization, and the factors by which it is influenced, are currently topics of investigation. Factors that affect repolarization include genetic mutations, which encode a variety of Long QT Syndromes (Keating and Sanguinetti, 2001; Ackerman, 1998; Perkiomaki et al., 2002), metabolic diseases (Bernardi et al., 1998; Veglio et al., 2004), electrolyte imbalances (Yelamanchi et al., 2001), sex (Ebert et al., 1998; Pham and Rosen, 2002), medications that prolong repolariza-

tion (Leatham et al., 1993; Hohnloser, 1997), changes in autonomic tone (Magnano et al., 2002; Bexton et al., 1986; Huang et al., 1992), and potentially many others as yet undescribed. Autonomic tone has been suggested as a significant determinant of QT interval duration and has both rate dependent and rate-independent effects on repolarization (Boyett and Jewell, 1980). Both the sympathetic and parasympathetic nervous systems' contributions to ventricular repolarization have been studied extensively. These contributions have been investigated in relation to circadian rhythm (Hohnloser et al., 1993; Marfella et al., 2003), during pharmacologic manipulations (Cuomo et al., 1997; Yee et al., 2000), during heart rate control via atrial pacing

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(Ahnve and Vallin, 1982; Guss and Kastor, 1976), and in patients with inherited Long QT Syndromes (Ackerman et al., 2002; Shimizu et al., 1997). As a whole these studies have generated disparate results, often with the same manipulation of the system having opposite effects in separate investigations (Lecocq et al., 1989; Cappato et al., 1991). These conflicting results could be due to a variety of factors including study design, dose and route of administration of compounds utilized to modulate the autonomic system, the correction factor employed with associated errors (Aytemir et al., 1999; Malik, 2002), or the influence of as yet undetermined factors other than or in addition to autonomic tone on cardiac repolarization.

Our laboratory developed the AV-node ablated His bundle-paced dog model to study the effects of drugs on cardiac repolarization independent of heart rate (Olivier et al., 2003; Sanders et al., 2004; Nolan et al., 2006). The model is also ideal for studies of the rate-independent effects of autonomic tone on cardiac repolarization.

Previous unpublished data have established a significant degree of rate-independent QT interval variability in the His bundle-paced dogs; it was theorized that change in autonomic tone was responsible for these QT interval fluctuations. Dogs exhibit abrupt and profound shortening of ventricular repolarization times in response to event stimuli that included entries into the vivarium, feeding, and cleaning. These entries into the vivarium resulted in excitatory behavior in the dog colony; dogs displayed behavioral evidence of arousal including rapid movement in their enclosures, panting and vocalization. Given the widely accepted view that the autonomic nervous system plays a role in the modulation of QT interval (Verrier and Lown, 1981; Hohnloser and Klingenhoben, 1994; Verrier and Antzelevitch, 2004), we sought to explore the rate-independent effects of sympathetic and parasympathetic blockade on event-related ventricular repolarization shortening in the His paced dog. We hypothesized that either sympathetic or parasympathetic blockade would either modify or potentially eliminate the repolarization shortening observed in His paced dogs following excitatory stimuli.

2. Materials and methods

2.1. Statement on use and care of animals

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Surgical methods

Six male beagle dogs weighing between 8 and 16 kg and of 22 to 42 months of age were selected for this investigation. Dogs were obtained from Marshall Farms (North Rose, NY) and were instrumented according to previously reported methods (Sanders et al., 2004). Briefly, dogs were anesthetized with 4–8 mg/kg of intravenous propofol and an inhaled

mixture of isoflurane in oxygen (Bedford Laboratories, Bedford, OH). A radiofrequency ablation catheter was advanced to the AV node and radiofrequency energy was applied to induce complete and permanent AV dissociation, as demonstrated on a continuous multi-lead ECG as P-waves and wide QRS complexes with no temporal relationship. A temporary ventricular pacing lead and external stimulator was used to stabilize the dog for the surgery to follow. Through a right thoracotomy, a custom made active fixation pacing lead was inserted into the His Bundle. This unipolar lead was tunneled to a mid-scapular subcutaneous pocket and connected to an implantable programmable pacing generator (Medtronic, Minneapolis, MN). Buprenorphine (VPL, Phoenix, AZ) was administered intramuscularly at 0.2 mg/kg immediately postoperatively and at 8 and 20 h postoperatively to provide analgesia. Dogs were allowed a minimum two-week recovery period before inclusion in this study.

2.3. Experimental methods

Study dogs were determined to be clinically normal by physical exam, complete blood counts and serum biochemistry analysis. Animals were housed individually in stainless steel runs. An automatic timing device provided an alternating 12 hour cycle of light and dark (6 am to 6 pm/6 pm to 6 am). Food was offered to the animals daily at approximately 7:00 am and water was supplied ad libitum via an automatic system.

Doses of 10 mg/kg of propranolol PO or 3 mg/kg of atropine methyl nitrate IV (Sigma Chemical Company, St. Louis, MO) given twice daily every 10 h were chosen to attenuate potential autonomic effects on ventricular repolarization. The selected doses were based on literature reviews (Heinzow et al., 1987; Tse et al., 1980; Albanus et al., 1969; Winbladh, 1973; Brorson et al., 1981) and unpublished pharmacokinetic/pharmacodynamic modeling designed to provide exposures that remained above the efficacious plasma concentration for beta- and parasympathetic blockade throughout the 24 hour collection period (Dawson et al., 1984; Vicenzi et al., 1995). The drugs were administered in a four-period crossover design with empty gelatin capsules given as control for the propranolol treatment and the intravenous saline vehicle given as control for the atropine methyl nitrate treatment. To attain steady-state plasma concentrations of the drugs prior to data collection, the drugs were administered at 24, 14 and 4 h prior to the start of data collection and a final dose was given 6 h after the start of data collection. Data

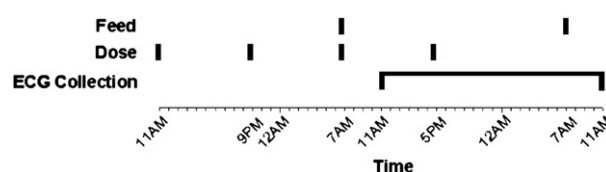


Fig. 1. Order of events before and during data collection.

Table 1
Mean RT interval change in His bundle-paced dogs following 5 pm and 7 am stimulus

Treatment	Change in RT interval (ms) 5 pm	<i>N</i>	Change in RT interval (ms) 7 am	<i>N</i>
Control	-18.5±6.7	6	-30.1±13.7	6
Propranolol	-14.7±8.1	5	-17.4±8.6	5
Vehicle	-20.5±11.8	6	-33.1±8.5	4
Atropine	-6.4±11.6	6	-14.1±9.2	6

Dogs were maintained at a His bundle-paced ventricular rate of 80 bpm. RT values represent means±SD and are expressed in milliseconds.

collection began at 11 am and concluded at 11 am the following day. The event stimulus was provided by investigators entering the room at 5 pm to administer the final treatment and at 7 am the following morning to feed the dogs and clean their runs. A period of at least 14 days for drug elimination was permitted between treatments.

The dogs' heart rates were maintained at 80 bpm. Animals were fitted with jackets, surface ECG electrodes, and radio-telemeters. A Lead II electrocardiogram was recorded for the continuous 24 hour period. The signal was amplified and digitally stored at a sampling rate of 1000 Hz. Fig. 1 gives a pictorial representation of the events occurring during the experiment.

2.4. Data analysis

The EMKA system (EMKA Technologies, Paris, France) was used for data acquisition and analysis. All interpretable ECG complexes from epochs of interest were analyzed and one-minute means were generated. Mild septal pre-excitation can be evident for His bundle-paced dogs complicating precise identification of the beginning of the QRS complex. Therefore, RT intervals defined as the time between the peak of R-waves and the end of T-waves are routinely substituted for the traditional QT measurement in this model. Automated ECG analyses were conducted using the EMKA derived algorithm. The peak of the R-wave and the end of the T-wave were initially identified on a sample beat as the zero crossing of the first derivative of the ECG signal. This "library" beat was used as a template for EMKA's shape-based algorithm to identify R-peak and T-end for all remaining beats. Complexes with no distinguishable end of T or with

superimposition of the P and T-waves were excluded from analysis. Typically 5–10 complexes each minute were excluded due to the above, and the one-minute means were generated from the remaining 70–75 beats.

One minute of ECG data were sampled every 15 min and these means were averaged to generate hourly means for the 24 hour data collection period.

2.5. Statistical analysis

Statistical analyses were conducted on RT interval. RT intervals were calculated hourly for the 6 hour period prior to each of the two stimuli. The average of the 6 hourly RT intervals preceding administration of the test articles was used as the baseline value for statistical comparisons. For the test of the effect of each of the two autonomic blockers, a grouped (control versus blocker) repeated measures ANOVA was performed with two repeats (baseline and 5 pm RT intervals). An effect of the blocker was denoted by a statistically significant group-time interactive effect. Paired *t*-tests were used to compare the mean RT interval shortening between the 5 pm and 7 am stimulus for each of the two treatments. Differences were considered significant at $p < 0.05$.

3. Results

Both the 5 pm and 7 am stimuli resulted in the desired and expected behavioral outcome; dogs displayed evidence of excitation including rapid movement in their enclosures and vocalization. Dogs treated with atropine methyl nitrate also demonstrated clinical evidence of efficacious parasympathetic blockade, including mydriasis, dry mucus membranes and a slight decrease in food intake noted by investigators. These signs persisted for up to two days after the conclusion of data collection. No dogs demonstrated clinical signs following treatment with control, vehicle or propranolol. Placebo treated animals (control and vehicle groups) had a considerable degree of RT interval shortening in response to stimulus (Tables 1 and 2; Fig. 2), ranging from 18.5 to 33.1 ms. RT shortening was always greater during the 7 am stimulus than at 5 pm, however, this was not a significant difference for any of the groups ($p=0.14$ for control animals, $p=0.27$ for the vehicle group, $p=0.16$ for atropine, and $p=0.46$ for propranolol

Table 2
Mean RT interval in His bundle-paced dogs preceding and following 5 pm and 7 am stimulus

Treatment	Mean RT interval (ms) 11 am–4 pm	<i>N</i>	Mean RT interval (ms) 5 pm	<i>N</i>	Mean RT interval (ms) 1 am–6 am	<i>N</i>	Mean RT interval (ms) 7 am	<i>N</i>
Control	211.8±4.3	6	193.3±5.3	6	214.5±3.9	6	184.4±4.6	6
Propranolol	211.6±4.7	5	196.0±5.9	5	212.8±4.3	5	194.6±5.1	5
Vehicle	209.7±4.3	6	187.7±4.4	6	213.2±4.3	4	180.1±8.2	4
Atropine	209.1±4.3	6	202.7±4.4*	6	208.7±3.5	6	194.0±6.7*	6

Dogs were maintained at a His bundle-paced ventricular rate of 80 bpm. RT values represent means of the 6 h preceding stimulus±SEM and the mean RT value during stimulus and are expressed in milliseconds.

* $P < 0.05$ compared with vehicle (atropine methyl nitrate).

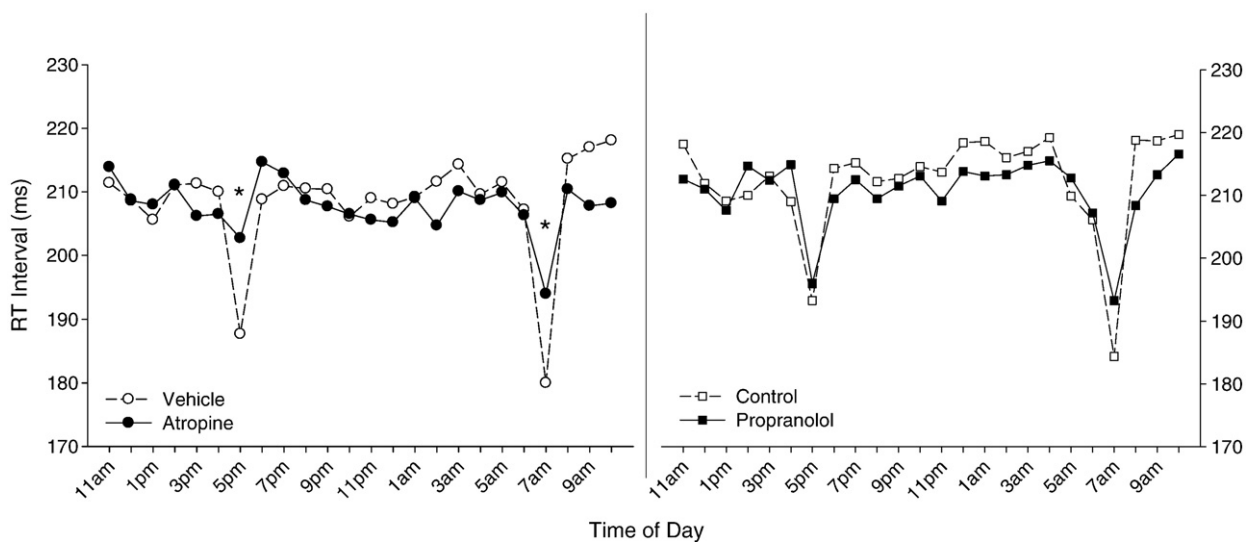


Fig. 2. Mean RT interval of His bundle-paced dogs. Investigators entered the room at 5 pm and at 7 am the following morning. Dogs were maintained at a His bundle-paced ventricular rate of 80 bpm. RT values represent means and are expressed in milliseconds. $N=6$ dogs for all control and atropine data, 5 dogs for propranolol data, and 4 dogs for vehicle data at 7 am. * $P<0.05$ compared with control (propranolol) or vehicle (atropine methyl nitrate).

treated dogs). Treatment with atropine attenuated the degree of RT interval shortening when compared with the vehicle group. At the 5 pm stimulus, the mean RT interval was significantly longer for the atropine treatment than for vehicle (202.7 ± 4.4 ms following atropine; 187.7 ± 4.4 ms following vehicle, $p=0.03$). There was no significant difference in RT interval duration between the propranolol and control treatments (196.0 ± 5.9 ms versus 193.3 ± 5.3 ms respectively, $p=0.51$). At the 7 am stimulus, the mean RT interval for the atropine treatment was also significantly longer than the vehicle treatment (194.0 ± 6.7 ms for the atropine treatment and 180.1 ± 8.2 ms for the vehicle treatment; $p=0.01$). There was no significant difference in RT interval seen for the propranolol and control groups at this same time point (194.6 ± 5.1 ms versus 184.4 ± 4.6 ms following control, $p=0.12$).

4. Discussion

The influence of autonomic tone on ventricular repolarization has been investigated extensively, with the wide variety of study designs, doses of autonomic modulators administered, and the correction factors employed generating often inconsistent results. Our use of classical antagonists in doses that likely ensure autonomic blockade, in a model that eliminates the need for QT correction factors, resulted in data that clearly demonstrate the important role of vagal withdrawal in ventricular repolarization shortening.

This study is unique in using an animal model that eliminates variations in HR and the need to apply correction factors when examining rate-independent repolarization variability. As reported in King et al. (2006), correction factors are fraught with errors, the best fixed parameter formula introducing an overcorrection of 10.8 ms and Bazett's formula introducing an overcorrection of as much as 67.9 ms to QT interval data. Errors of 6 ms were still introduced by linear

regression methods, making any data including corrected QT intervals potentially suspect. With this preparation we are able to avoid this issue.

Our model clearly demonstrates that when dogs become excited there is a significant uncorrected rate-independent shortening in the RT interval, as large as 33 ms in untreated animals. This degree of repolarization variability mirrors that seen in pacemaker patients by Bexton et al. This patient group demonstrated a mean QT interval shortening of 37 ms at a paced rate of 70 bpm.

We hypothesized that the shortening of RT interval observed during interactions with the dogs was a consequence of either increased activity of the sympathetic nervous system or decreased activity of parasympathetic nervous system. We tested these hypotheses by the use of the classic efficacious and specific antagonists, administered at doses known to provide complete muscarinic and beta-adrenergic blockade for 24 h in dogs (Heinzow et al., 1987; Tse et al., 1980; Albanus et al., 1969; Winbladh, 1973; Brorson et al., 1981; Dawson et al., 1984; Vicenzi et al., 1995). Therefore we likely provided complete muscarinic and beta-adrenergic blockade for the duration of the experiment. These results suggest that parasympathetic withdrawal is the primary factor responsible during both awake hours (5 pm) and in the transition from deep sleep to the awake state (7 am) in the facilitation of RT interval shortening following an excitatory stimulus. Our data did show a difference in the degree of RT shortening in the presence of beta blockade (-30.1 ± 13.7 ms for the control group and -17.4 ± 8.6 for propranolol treated animals), but this difference was not statistically significant. Therefore, an increase in sympathetic tone could have some effect on RT interval change during waking hours; however, in this study it plays no statistically significant role.

The conclusions of these studies are supported by the observations of Magnano et al. who reported that infusions of

isoproterenol resulted in less QT interval shortening than either exercise or atropine infusion. Subjects underwent bicycle exercise, or atropine or isoproterenol infusions and HR/QT data were modeled for each intervention and compared. As heart rates of the subjects increased, QT interval was longer during isoproterenol treatment in comparison to exercise or atropine treatment, which had similar repolarization durations. The investigators concluded that the similarity in HR/QT behavior between exercise and atropine treatments reflected the importance of vagal inhibition, potentially to a greater degree than sympathetic stimulation, in facilitation of QT interval shortening. Lecocq et al. examined the QT/RR relationship in healthy volunteers at rest, during dynamic exercise and after injections of isoproterenol or atropine or treatment with propranolol. They also concluded that physiologic QT-RR adaptation is mainly under parasympathetic control. Hohnloser et al. (1993) followed a group of patients referred for ventricular ectopic activity and performed a series of 24 hour ECG recordings before and after the administration of the class III antiarrhythmic d-sotalol. Interestingly, the patient group retained the circadian pattern to their heart rate and QT interval even in the presence of efficacious beta blockade, suggesting that sympathetic tone alone is not responsible for the entirety of circadian fluctuation in ventricular repolarization.

The attenuation of RT interval shortening following muscarinic blockade observed during excitatory stimulus may be a direct effect of atropine on the ventricular myocardium. However, if this were the case one might expect atropine treated animals to have a longer or shorter RT interval than vehicle treated animals throughout the experiment. Potentially an indirect mechanism is responsible for the observed attenuation of RT interval shortening. In human ventricles, stimulation of M2 receptors by acetylcholine causes indirect negative inotropic effects that can only be demonstrated if basal force of contraction has been previously enhanced by cyclic AMP-elevating agents such as epinephrine and norepinephrine (Brodde et al., 2001). In this study the excitatory stimulus may serve to generate the surge of catecholamines necessary to reveal atropine's effect.

This study has two major limitations. First, propranolol and atropine were not given concurrently in order to examine the effects of complete autonomic blockade on RT interval shortening. Additionally, propranolol, while blocking both beta-1 and beta-2 receptors, has no activity against alpha-adrenergic receptors, leaving their effects on ventricular repolarization unexamined. Truly complete sympathetic blockade would be better achieved by the concurrent administration of propranolol and a long-acting alpha antagonist (Hoffman, 2001).

In conclusion, parasympathetic withdrawal is the primary factor responsible in the facilitation of rate-independent repolarization shortening following an excitatory stimulus in dogs. The attenuation of RT interval shortening following atropine treatment may be a direct effect, or an indirect effect requiring an excited state to become apparent. The use of a

model that eliminates the need to apply correction factors to repolarization indices lends additional clarity to the complex role of the autonomic nervous system on cardiac repolarization.

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